Ady2p is essencial for the acetate permease activity in the yeast *Saccharomyces cerevisiae*

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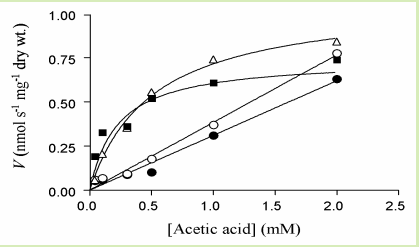
Several yeast species, including *Saccharomyces cerevisiae*, are able to utilize acetic acid as a sole carbon and energy source under aerobic conditions (Barnett *et al*., 1990), acetate being a normal and frequent end-product of fermentation (Flores *et al*., 2000). When cells of *S. cerevisiae* are grown on respiratory substrates, activity for at least two monocarboxylate proton symporters are found, with differences in their mechanisms of regulation and specificity (Casal *et al*., 1996). A lactate–pyruvate-acetate-propionate transporter, induced in lactic or pyruvic acid-grown cells, is encoded by the gene *JEN1* (Casal *et al*., 1999). In addition to Jen1p, another permease, which accepts acetate, propionate or formate, is present in cells grown in non-fermentable carbon sources (Casal *et al*., 1996; Makuc *et al*., 2001). To identify new genes involved in acetate uptake in *S. cerevisiae*, an analysis of the gene expression profiles of cells shifted from glucose to acetic acid was performed. This strategy allowed us to identify the membrane protein Ady2p as an essential component of the acetate active transport in these conditions and a valuable candidate as a new acetate transporter in yeast.

Acetate is transported by a mediated transport system

Glucose-grown cells → Shift to YNB-acetic acid medium, pH 6.0



After 4 h induction
○ W303-1A (*JEN1*)
● W303-1A (*jen1Δ*)

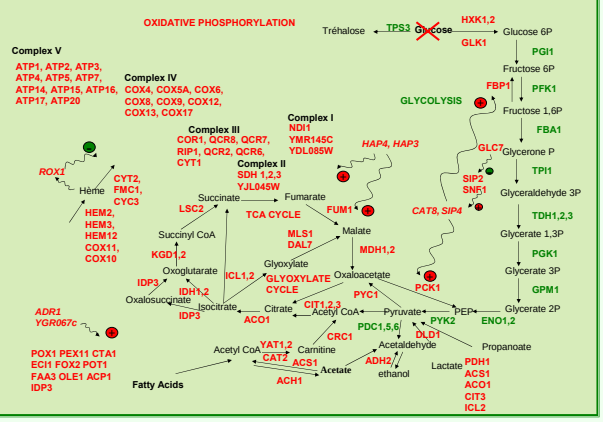
After 6 h induction
△ W303-1A (*JEN1*)
■ W303-1A (*jen1Δ*)

➢The activity of the acetate permease was present only after 6 h of induction

➢The deletion of *JEN1* did not affect the ability of the cells to transport acetic acid by a mediated mechanism

Setting up the best conditions for microarray analyses

Glucose-grown cells → Shift to acetic acid medium, 4 h



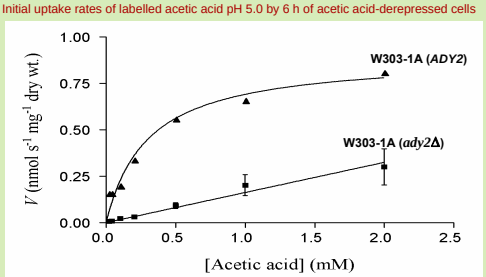
Expression changes in genes encoding known or putative transporters after 4h in acetic acid

	Fold induction after 4 hours in acetate	Substrate
Mitochondrial transporters		
TCA cycle intermediates		
EFC1	30	Succinate/fumarate antiport
CRC1	22	Carnitine
ODC1	9.3	Oxoglutarate
OAC1	- 5.1	Oxaloacetate
Oxydative phosphorylation products		
AAC1, AAC3, PET9	8.2 to 8.6	ATP/ADP antiport
MIR1	12	Inorganic phosphate
Others		
ACP1	3.0	Fatty acids precursors
DIC1	- 3.0	Dicarboxylates
Plasma membrane transporters		
Carboxylic acids		
JEH1	42	Lactate, propionate, acetate
MCH3 ,MCH4, MCH5	-2.2 to -3.7	unknown
Amino acids and carnitine		
AGP1	2.3	Asparagine, glutamine, cysteine, etc.
TAT1	8.1	Valine, leucine, tyrosine, etc.
MUP1	3.4	methionine
AQP2	5.7	carnitine
Hexoses		
HXT1, HXT2, HXT3, HXT4, HXT5, HXT6, HXT7, HXT8	2 to 6.3	Hexoses
Inorganic ions		
SMF2	2.5	Manganese
CTR1	4.2	Copper
FET3, FTR1	2.1/3.1	Iron
ENA1, ENA2, ENA5	2.3	Sodium, lithium
Unknown membrane proteins		
ADY2	27	unknown
FUN34	9.8	unknown
ATO3	2.6	unknown

➢Genes *YCR010c/ADY2*, *YNR002c/FUN34* and *YDR384c/ATO3*, homologous to the *GPR1* gene of *Yarrowia lipolytica* were clearly activated

ADY2/YCR010c is an essencial gene for the mediated transport of acetic acid in *S. cerevisiae*

Initial uptake rates of labelled acetic acid pH 5.0 by 6 h of acetic acid-derepressed cells

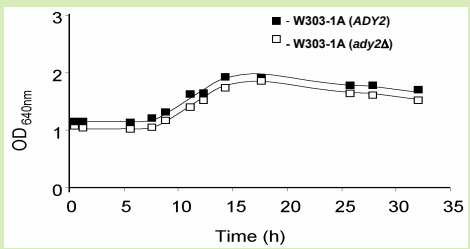


➢ *ADY2*-deleted strain (■) exhibited a dramatic decrease in the activity of the acetate premease when compared to the wt strain (▲)

➢ In the *ADY2*-deleted strain acetic acid crossed the plasma membrane by a simple diffusion mechanism

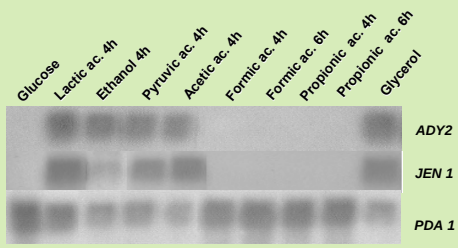
Adaptation of *S. cerevisiae* W303-1A and *ady2Δ* strains to acetic acid (0,5%, pH 6.0)

Cells were cultivated in glucose and transferred to a medium containing acetic acid (0,5%, pH 6.0)



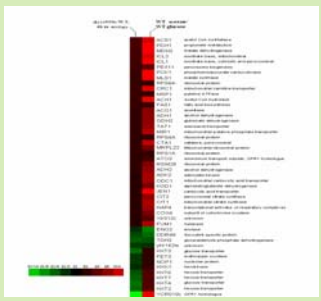
➢ Cells of the *ady2Δ* have a normal growth in acetic acid when compared with the wild type strain

Expression of *ADY2* in different carbon sources



Northern-blot analysis of *JEN1* and *ADY2* transcripts from W303.1A strain. Total cellular RNA was prepared from cells growing exponentially in YNB media containing different carbon sources

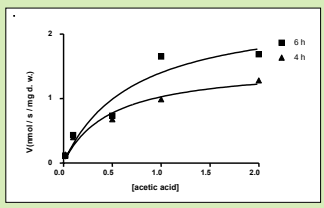
Comparison of gene expression profiles between W303-1A and *ady2Δ* strains



After 4 hours in acetate, the *ady2Δ* strain does not have a dramatic difference in the global transcriptional response compared to the wild type suggesting that *ADY2* is not a key regulator of the transcriptional response to acetic acid

Analysis of acetate uptake in *S. cerevisiae ady2Δ* containing the gene *ADY2* in the plasmid p416GPD

YNBgluc → YNBace



	4h	6H
K_m (mM)	0.48	0.72
V_{max} (nmols^{-1} mgdw^{-1})	1.5	2.4

Presence of a mediated transport system for acetic acid

The transcriptional adaptation of yeast cells to a shift from glucose to non-toxic conditions of acetic acid was characterized. These conditions of metabolic shift revealed to be very powerful for functional assignment of unknown proteins and for the study of mechanisms like translation regulation or mitochondria biogenesis.

➢***ADY2* gene is an essential component of acetate transport system in *S. cerevisiae***

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Barnett, JA. *et al*., 1990. *Yeast. University Press*; Casal, *M. et al*., 1996. *Microbiology*, 142: 1385-1390; Casal, M. *et al*., 1999. *J. Bacteriol*., 181: 2620-2623; Flores, CL. *et al*., 2000. *FEMS Microbiol. Rev*., 24: 507-529; Makuc, J. *et al*., 2001. *Yeast*, 18: 1131-1143.